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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (1): 98-104, 1999 *Research Article*

Use of Genome-specific Satellite DNA Sequences to Study the Nature and Extent of Genome Differentiation in Evolution of *Lathyrus*

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Abstract

A cloned restriction fragment of a highly repetitive satellite DNA sequence from *L. tingitanus* was used as a molecular probe to study the nature and extent of genome differentiation in 22 *Lathyrus* species and 17 natural population of *L. sativus*. Sequences homologous to the satellite DNA were identified in the genomes of all *Lathyrus* species and natural population of *L. sativus*. However, substantial base sequence divergence was found in sequences homologous to the satellite DNA in different species. This was evident in the large variation in restriction fragment lengths. Similarity indices calculated from the restriction fragment length distributions were compared with the known taxonomical relationship between species established from morphological and molecular studies. The degree of genome differentiation was significantly small between population when compared to the greater variation between species.

Introduction

The genomic classification has been based largely on the analysis of chromosome pairing behaviour in inter specific hybrids. Recently molecular markers have been utilized to study the genome differentiation in various plant species. Both single-copy (Song *et al*., 1988; Bonierbale *et al*., 1988) and repetitive DNA sequences (Aswidinnoor *et al*., 1991; Anamthawat-Jonsson and Heslop-Harrison, 1993 and Mawal *et al*., 1995) have been used. Repetitive DNA is the major determinant of plant chromosome structure and of the nucleotype. The nucleotypic variation in plants is mainly brought about by amplification and deletion of dispersed repeat elements, whereas DNA variation by satellite turnover is "recent icing on the cake" (Schweizer *et al*., 1990).

In *Lathyrus* upto 70 percent of the total DNA is made up of repetitive sequences which account for most of the DNA variation within this genus (Narayan and Rees, 1976). The repetitive components range from almost identical satellite DNA sequences to families of repetitive sequences which show substantial divergence within and between them. The remainder of the genome is made up of non repetitive sequences (Narayan and Rees, 1976). Narayan (1985) used satellite DNA as a template to transcribe tritium labelled complementary RNA (C-RNA) of denatured chromosomal DNA of *L. tingitanus* and other related species. The results showed that the basic repeat unit of the *L. tingitanus* satellite DNA is present in all species investigated. Restriction analysis and hybridisation of ^{32}P labelled satellite DNA to the Southern blots of restricted genomic DNA of *L. tingitanus* has shown that the satellite DNA can be distinguished as a restriction fragment (Tiller, 1991). Different restriction enzymes generated unique restriction bands differing in molecular weights. In this research paper molecular techniques were utilized for determining the genomic relationships of *Lathyrus* species at DNA level. Highly repetitive DNA sequence of a 1.8 kilobase (kb) *Eco*RI fragment selected as a molecular probe. The objective was to study the distribution and divergence of this sequence in the genomes of 22 related *Lathyrus* species and 17 accessions of *L. sativus* (Var. 5807, Var. 8246, K447, K482, K282, K271, K505, K298, K661, K256, K290, K663, K258, K397, K554, K333 and K401).

Materials and Methods

The research involved 22 species from genus Lathyrus and 17 accessions of *Lathyrus sativus* which occur as natural populations in Pakistan and Ethiopia. The seeds of *L. sativus* varieties were obtained from Agriculture Research Institute Islamabad, Pakistan and Agriculture Canada Research Station, Minitoba Canada. *Lathyrus* species collected from Research institutions and Botanical gardens were maintained at the University of Wales, Aberystwyth, UK since early 1974.

DNA isolation and purification: Total DNA was extracted from freshly harvested leaves using a method as described by Murray and Thompson (1980) with some modifications. Plasmid DNA was isolated by several methods using techniques in laboratory manual Sambrook *et al*. (1989) and by He *et al*. (1990).

Cloning of *L. tingitanus* repetitive DNA sequences: The purified DNA of *L. tingitanus* was restricted with *Eco*RI, *Bam*HI, *Hae*III, *Taq*I, *Alu*I and *Msp*I restriction enzymes under the conditions described by the suppliers Bethesda Research Laboratory (BRL). The DNA fragments were separated by agarose gel electrophoresis at about 3V/cm for 4 hours on 1 percent agarose Type I using λ DNA restricted with *Hind*III as a standard molecular weight marker. All enzymes produced restriction patterns with prominent bands as visualised with ethidium bromide and

viewed under ultraviolet light. A 1.8 kb *Eco*RI fragment was among the most intense band, presumably the most abundant repetitive sequence in the genome.

Ligatoin and transformation: The vector DNA (pUC-18) was linearized by cleaving a unique restriction site in the polylinker region with *Eco*RI. The linearized vector and satellite DNA cut with *Eco*RI restriction enzyme, produced compatible sticky ends. The DNA fragments to be cloned (1.8 kb *Eco*RI) was eluted from low melting point agarose gel using the method suggested by Heery *et al*. (1990). Ligation reaction was performed in a reaction volume of 20 μl. For optimum ligation the vector and insert ends were used in an equi-molar ratio. The vector and insert DNA fragments were ligated using T4 DNA ligase. The reaction was incubated overnight at 16°C. The preparation and transformation of *E. coli* cells were performed as described by Sambrook *et al*. (1989).

Screening and amplification of the recombinant colonies: The *E. coil* host strain transformed with recombinant pUC18 plasmid vector produced white colonies on the X-gal plates. The transformants with functional galactosidase gene metabolised the X-gal and produced blue colonies. Identification of the recombinant pUC colonies was done by both ampicillin resistance and blue/white colony selection. The plasmids DNA isolated from the white recombinant clones were further analyzed by polymerase chain reaction (PCR) to confirm the size of the inserted DNA fragment.

PCR was performed using a Perkin-Elmer thermocycler. The method was originally devised by Mullis and Faloona (1987). Amplification reaction was proceeded in volumes of 50 μl reactions. The reaction mixture comprised of 5 μl of 10X PCR buffer (Advanced Biotechnology), 5 μl of 2mM dNIP's mixture, 3 μl of 25 mM $MgCl_2$, 20 pmoles of M13 universal primers forward (5′-GTA AAA CGA CGG CCA GT-3′) & reverse (5′-AAC AGC TAT GAC CATG-3'), 2ng of pUC template DNA and 1 unit of *Taq* DNA polymerase. The reaction volume was made upto 50 μl with HPLC grade water. The contents of tubes were mixed and spun briefly to consolidate and a drop of mineral oil was overlaid in each tube. The reaction tubes were placed into wells in the thermal cycler. Cycling parameters for insert amplification for the reaction was 1 cycle of 94°C for 5 minutes, 55°C for 2 minutes, 72°C for 2 minutes; 30 cycles of 94°C for 1 minute, 55°C for 1 minute, 72°C for 2 minutes and finally 1 cycle of 72°C for 7 minutes. The PCR product was detected by gel electrophoresis using standared size marker (λ DNA *Hind*III restricted).

Southern blot hybridisation and Autoradiography: Restriction enzymes digests of genomic DNA samples were electophoresed. Southern blotting and hybridisation was done using standard methods Sambrook *et al*. (1989). Cloned inserts were amplified by PCR and then labelled with [^{32}P] dCTP using the random primer method (Feinberg and Vogelstein, 1983).

To develop autoradiograph the dried filters were wrapped in Saran wrap and exposed to X-ray film (Amersham Hyperfilm TM-MP or Kodak X-Omat AR) in a lead backed cassette (Genetic Research Instrumentation Ltd.). Two intensifying screens (Cawo special) were used to increase the definition of the autoradiographs. The cassettes were placed at -70°C in a freezer and the duration of the exposure varied with the specific activity of the DNA probe. After exposure, the cassettes were brought to room temperature and developed for 3 minutes 1:5 dilution of GBX developer and 1:5 dilution of GBX fixative for 6 minutes respectively. The x-ray films were washed in running tap water 15-25 minutes.

Results

Identification and characterisation of cloned *L. tingitanus* satellite DNA sequences: The genomic DNA of *L. tingitanus* was restricted with six restriction endonucleases as described earlier. As would be seen in Fig. 1a restriction profiles differ for different restriction enzymes. Such differences suggest extensive modulation in the amount and sequence organisation of the repetitive families. Most of the repeated sequences in this species are known to be of interspersed Afzal (1989). It was considered possible that several of the bands visible in restriction digest might be closely related or form part of large repeating unit, a relationship that could not be established from ethidium bromide staining alone. A 1800 by fragment generated by *Eco*RI was cloned in pUC vector and used as a molecular probe to study the distribution and divergence in the genome of *L. tingitanus* and in the genomes of related species. This will be referred to as E-1800 sequence.

Insert sequences in recombinant pUC vectors when selectively amplified using M13 universal primers in a PCR reaction. From few white colonies four were selected for PCR amplification reaction. Out of the four recombinant colonies used three (lanes 1, 2 and 4) showed insert sequence lengths of 1664 bp, 1733 bp and 1800 by respectively (Fig. 2). The colony in lane 3 showed a slightly less insert size (1400 bp). The positive recombinant colonies 2 and 4 represent highly repetitive DNA sequences which make 6-7 percent the total DNA of *L. tingitanus* (Naravan and Durrant, 1983). Of the two positive clones, the one with 1800 by insert sequence was selected for further investigation.

Organisation and distribution of E-1800 sequences in *L. tingitanus* genome: Genomic DNA of *L. tingitanus* was restricted with six restriction enzymes (*Eco*RI, *Bam*HI, *Hae*III, *Taq*I, *Alu*I and *Map*I), electrophoresed and Southern blotted onto Hybond N^+ filters. The insert DNA of clone 4 was PCR amplified. The amplified fragment was eluted from agarose gel as described earlier and labelled with α-^{32}P dCTP by random primer labelling method. The purified probe was hybridised to Southern blots of *L. tingitanus* DNA restricted with different enzymes. The autoradiograph

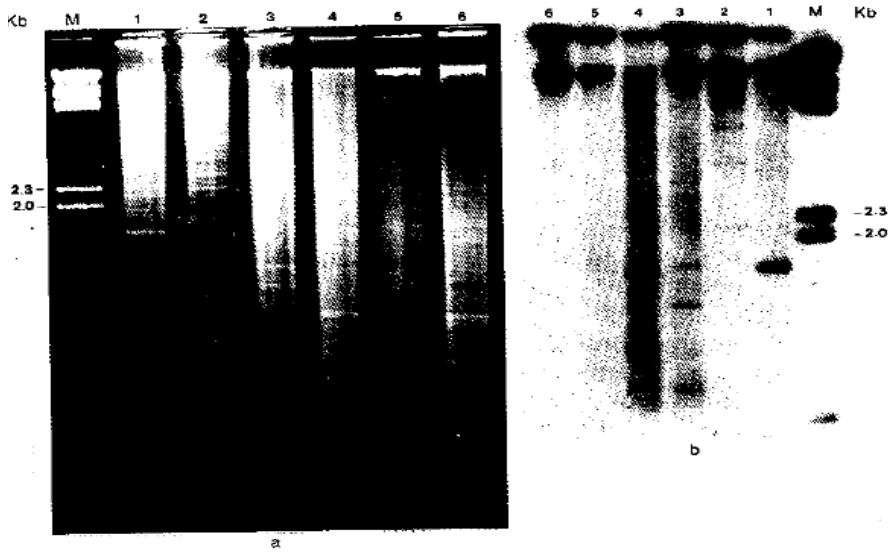

Fig. 1: (a) Restriction patterns of *L. tingitamis* genomic DNA digested with six different endonucleases. 1. *Eco*RI (Arrow indicates the 1800 by fragment), 2 *Bam*HI, *Hae*III, 4. *Taq*I, 5. *Alu*I, 6. Msp. M. Molecular size marker (λ DNA restricted with *Hind*III) (b) Autoradiograph shows the distributin of sequences homologous to E-1800 satellite DNA within the genome of *L. tingitanus* digested with six different restriction endonucleases as in Fig. 1a.

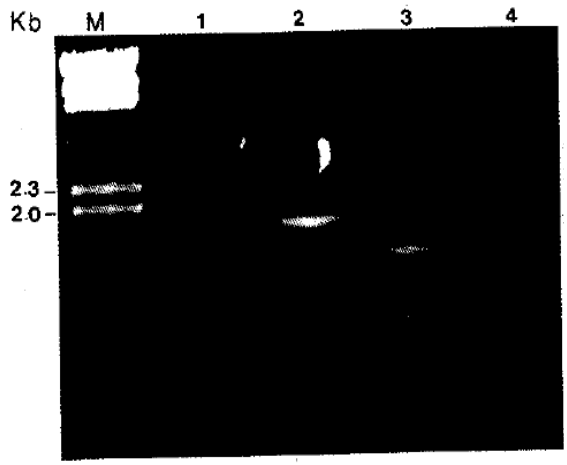

Fig. 2: PCR amplified E-1800 sequences from positive clones lanes 1, 2 and 4 insert sequence lengths 1664 bp, 1733 bp and 1800 by respectively. The colony in lane 3 showed a slightly less insert size 1400 bp.

showed differences between restriction profile, the molecular weights of the restriction fragments ranged from 600 by to 6.9 kb (Fig. 1 b). However, restriction bands of identical molecular weights (1800 bp) could be seen for *Eco*RI, *Hae*III and *Taq*I, lanes 1, 3 and 4 respectively. This could result from the tandem repetitiveness of the satellite DNA. The intensity of restriction bands in the autoradiograph was low in lanes 5 and 6 where *Alu*I and Msp I respectively. The differences in fragment lengths, and generated by different restriction enzymes, would suggests that the recognition sites are distributed differently for different enzymes in the satellite DNA.

Distribution of sequences homologous to E-1800 sequence in the genomes of 22 *Lathyrus* species: Of the 22 specie investigated the nuclear DNA of *L. cirrhosus* did not restrict with different restriction enzymes. This may be due to species-specific secondary metabolites that were no removed during the standard DNA extraction procedure would appear that *L. cirrhosus* requires a modifying extraction procedure. The 22 species, classified into six s generic sections (Kupicha, 1983), comprise a large variation in 2C nuclear DNA amounts ranging means from 10.76 in *L. angulatus* to 24.78 pg in *L. latifolius* (Narayan, 1985). Three restriction enzymes (*Eco*RI, *Barn*HI and *Hind*III) each recognising a 6 bp restriction site were used to restrict the genomic DNA of different *Lathyrus* species.

The autoradiograph (Fig. 3) shows the distribution sequences homologous to the E-1800 satellite DNA in the genomes of 21 species (*L. cirrhosus* did not produce restriction profile lane 21). The DNA samples were restricted with *Eco*RI. Species show differences in the distribution of sequences homologous to the satellite DNA. Number of fragments highlighted in a lane ranged from 2 to 8. In the homologous hybridisation with *L. tingitan* genomic DNA (Fig. 3 lane 1) a prominent 1.8 kb band highlighted. The remaining species however, showd variation in the lengths of fragments homologous to the satellite DNA. The lengths ranged from 1.6 kb to 8.8 kb (data not shown). Similar experiments were performed for

the 21 species using *Bam*HI and *Hind*III restriction enzymes. The molecular weight distributions of restriction fragments were different for *Bam*HI and *Hind*III for all species compared. The intensity of the restriction bands highlighted in the auturadiograph also varied. This could result from quantitative DNA differences in the highlighted bands or due to substantial base sequence divergence. The distribution of fragment lengths also shows a well defined periodicity which would normally be expected in the restriction profile of repeated DNA sequences.

Distribution of E-1800 satellite DNA and genome differentiation in *Lathyrus*: Analytical density gradient analysis in neutral CsCI did not readily reveal satellite DNA components in the genomes of most *Lathyrus* species (Narayan and Rees 1976). However nuclear DNA when coupled with actinomycin-D or silver, in different molar ratios, showed varying amounts of satellite DNA in the genomes of different species (Narayan, 1985). *In situ* hybridisation is not an efficient experimental method to survey the distribution of specific DNA constituents in the genomes of related species. In the present investigation the genomic DNA of 22 *Lathyrus* species was hybridised with the E-1800 satellite DNA using the method of Southern blot hybridisation. As would be seen in Table 1 the 22 species are from 6 different subgeneric sections of *Lathyrus*. The large genome size variation between species suggest substantial divergence in genome organization in evolution. Despite these differences the satellite DNA from *L. tingitanus* has hybridized with the genomic DNA of all species compared. This suggests that the basic repeat unit of the satellite DNA is present in the genomes of all species examined.

Species included in section *Lathyrus* are more similar taxonomically to *L. tingitanus* than species included in other sections. It was important to find whether, species included in section *Lathyrus* were more similar to *L. tingitanus* for restriction fragment length distribution of the satellite DNA than species included in other subgeneric sections. A comparison was therefore made of the similarity indices calculated by the formula as suggested by Nei and Li (1979). The F ratios and their means are given in Table 1 Species classified into section *Lathyrus* show several characters that are partially correlated which suggests that they show a similar trend in evolution (Kupicha, 1983). The mean similarity index is 0.5 for section *Lathyrus*. Species of section *Linearicarpus* show a mean similarity index of 0.49. Section Nissolia is monotypic with a single species *L. nissolia*. Section Aphaca is ditypic with two species *L. aphaca* and *L. stenolobus*. *L. nissolia* and *L. aphaca* show partial resemblance to species included in section *Linearicarpus* and section *Pratensis* respectively. They differ significantly from other species for most taxonomical characters. The similarity index for *L. nissolia* is 0.45 and for *L. aphaca* 0.38. Species included in section *Clymenum* have an intermediate value of 0.46.

In genus *Lathyrus* the distribution of 2C DNA amounts is discontinuous, species cluster into DNA groups. While species included in each DNA group are closely similar in genome size the group means are separated by a regular average interval of about four pg. This classification occur independently of the taxonomical relationships between species (Narayan, 1982, 1985). The discontinuity presumably relates to certain intrinsic constraints upon evolutionary changes in genome organization. Such discontinuous distributions are evident in *Vicia*, *Clarkia*, *Lolium* and *Nicotiana*. A similar similarity index analysis was carried out for species classified into different DNA groups. In Table 2 L. tingitanus is included in group 4 together with *L. hirsutus* and *L. tuberosus*. The mean F ratios for group 4 is not significantly different from the mean F ratios of other groups.

Table 1: Comparison of mean similarity index values calculated separately for different sections of *Lathyrus*.

Species/Sections	*Eco*RI F-ratio	*Bam*HI F-ratio	*Hind*III F-ratio	Means
1. Lathyrus				
L. tingitanus	-	-	-	-
L. cicera	0.33	0.36	0.28	0.32
L. sativus	0.28	0.54	0.28	0.36
L. odoratus	0.80	0.54	0.57	0.63
L. sylvestris	0.80	0.61	0.57	0.66
L. latifolius	0.80	0.61	0.57	0.66
L. gorgoni	0.50	0.44	0.40	0.44
L. hirsutus	0.66	0.44	0.50	0.53
L. heterophyllus	0.40	0.50	0.57	0.49
L. hierosolymitanus	0.80	0.44	0.66	0.63
L. annuus	0.66	0.44	0.33	0.47
L. tuberosus	0.40	0.44	0.28	0.37
Mean	0.58	0.48	0.45	0.50
2. *Aphaca*				
L. aphaca	0.28	0.60	0.28	0.38
3. *Clymenum*				
L. articulates	0.44	0.72	0.33	0.49
L. clymenum	0.44	0.60	0.25	0.43
L. ochrus	0.33	0.44	0.66	0.46
Mean	0.40	0.58	0.41	0.46
4. *Linearicarpus*				
L. inconspicuus	0.44	0.44	0.40	0.42
L. sphaericus	0.44	0.44	0.80	0.56
L. angulatus	0.50	0.72	0.28	0.50
Mean	0.46	0.54	0.49	0.49
5. *Nissolia*				
L. nissolia	0.50	0.54	0.33	0.45

(Similarity index was calculated as F = nXY/(nX + nY), where nXY is the number of bands present in both lanes. nX is the total number of bands in lane X and nY is the total number of bands in lane Y).

Table 2: Comparison of mean similarity index values calculated separately for different DNA groups of *Lathyrus*.

Species/Groups	*Eco*RI F-ratios	*Sam*HI F-ratios	*Hind*III F-ratios	Means
Group 1 (mean - 10.76 pg)				
L. angulatus	0.66	0.54	0.31	0.50
Group 2 (mean = 13.80 pg)				
L. annuus	0.33	0.44	0.33	0.36
L. hierosolymitanus	0.80	0.44	0.50	0.58
L. cicera	0.33	0.36	0.28	0.32
L. aphaca	0.28	0.60	0.28	0.38
L. articulatus	0.44	0.72	0.33	0.49
L. clyrnenum	0.44	0.60	0.25	0.43
L. ochrus	0.33	0.44	0.66	0,47
L. nissolia	0.66	0.54	0.33	0.51
L. sphaericus	0.40	0.44	0.80	0.54
L. inconspicuus	0.40	0.44	0.40	0.41
Mean	0.44	0.50	0.41	0.45
Group 3 (mean = 16.88 pg)				
L. sativus	0.28	0.54	0.28	0.36
L. odoratus	0.80	0.54	0.57	0.63
L. gorgoni	0.50	0.44	0.40	0.44
Mean	0.52	0.50	0.41	0.47
Group 4 (mean = 20.51 pg)				
L. tingitanus				
L. hirsutus	0.66	0.44	0.50	0.53
L. tuberosus	0.40	0.44	0.28	0.37
Mean	0.53	0.44	0.39	0.45
Group 5 (mean = 24.53 pg)				
L. heterophyllus	0.40	0.60	0.57	0.52
L. sylvestris	0.80	0.61	0.57	0.66
L. latifolius	0.80	0.61	0.57	0.66
Mean	0.66	0.60	0.57	0.61

The satellite DNA was also hybridised with the genomic DNA extracted from 17 varieties of *L. sativus*. The 17 varieties were ecotypes collected from Pakistan. The DNA was restricted with *Eco*RI. The restriction profiles were remarkably similar for the all 17 varieties. The greater differences seen in the restriction profiles between species is a function of the time elapsed since they diverged. Intraspecific differentiation between varieties is relatively recent on an evolutionary time scale. Therefore, the restriction profiles were very similar in the autoradiograph. Whereas interspecific differentiation is associated with strong reproductive barriers, interpopulation divergence within a species is not accompanied by reproductive isolation.

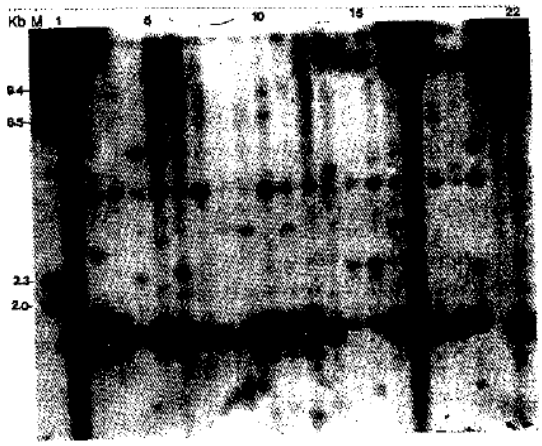

Fig. 3: Autoradiograph shows the distribution of sequences homologous to E-1800 satellite DNA in the genomes of 22 *Lathyrus* species. The DNA of each species was digested with *Eco*RI restriction endonuclease. Lanes are: M. Molecular size marker (A DNA restricted with *Hind*III), 1. *L tingitanus* 2 *L. angulatus*, 3. *L. articulatus*, 4. *L. nissolia*, 5. *L. cicera*, 6. *L. sativus*, 7. *L. odoratus*, 8. L. sylvestris, 9. *L. latifolius*, 10. *L. clymenum*, 11. *L. aphaca*, 12. *L. gorgoni*, 13. *L. hirsutus*, 14. *L. inconspicuus*, 15. *L. spaericus*, 16. *L. heterophllus*, 17. *L. tingitanus*, 18. *L. hierosolymitanus*, 19. *L. annuus*, 20, *L. ochrus*, 21. *L. cirrhosus*, 22. *L. tuberosus*. Lane 21 *L. cirrhosus* did not produce a restriction profile.

Discussion

Genome specifies the genetic make up of a species. The 2C nuclear DNA amount, haploid chromosome number and karyotype arrangement are constant within a stable species. Changes in genome organization creates reproductiv barriers that promote species divergence. The molecule processes determining the overall rate of genomic change include, saltatory amplification, deletion and conversion a DNA sequences. These changes are further compounded by nucleotide sequence divergence and inherent errors a genomic machinery (Schopf, 1981). Transposable genetic elements can alter genes and genomes by causing gross chromosomal rearrangements (inversions, translocation and deletions) which may result in a changed pattern of gene expression and alter linkage equilibria in populations (Finnegan, 1989).

It is assumed that the presence of satellite DNA sequence confers some selective advantage to the organis otherwise it is not possible to account for its spreadin throughout the population (Walker, 1971). The mechanis

of distribution of satellite DNA within and between the chromosome complements is difficult to explain particularly in terms of the degree of base sequence divergence found in many related species. It is logical to suppose that the satellite DNA sequences would have conferred a selective advantage, not to the organism as a whole, but to the chromosome carrying it. Once amplified within a chromosome, the satellite sequences would have spread to other chromosomes of the complement by translocations. Once it has moved to a new position in the complement, a large number of mutations could change the DNA composition.

When restricted genomic DNA of *L. tingitanus* and related species were hybridised with E-1800 satellite DNA the autoradiograph highlighted several restriction bands differing in fragment lengths. The large scale variation in restriction fragment length would suggest that the satellite DNA exits in multiple copies in the genome of *L. tingitanus* and the member sequences have diverged at least to a limited extent in evolution. The three restriction endonucleases generated different patterns of fragment length distributions homologous to satellite DNA sequences. The 21 species represent a two fold variation in genome size ranging from 10. 76 pg in *L. angulatus* to 24. 78 pg in *L. latifolius*. Quantitative and qualitative differences in the restriction profiles as highlighted in the autoradiograph would suggest extensive differentiation of the *Lathyrus* genome in evolution.

Despite these large scale differences between species, the restriction profiles of sequences homologous to the satellite DNA showed an element of periodicity. Such periodicities are common in the restriction profiles of repetitive sequences. A 3.6 kb restriction fragment is common to all species in the autoradiograph. This and other restriction fragment lengths, 5.4 kb, 7.2 kb and 9 kb approximately correspond to sequence lengths that are multiples of the 1.8 kb basic repeat. Random mutations in restriction sites could generate such periodicity in the restriction profiles. Unequal crossing over is another molecular mechanism that could cause differences in sequence lengths (Dover, 1982). Species included in a genus presumably have diverged from a common ancestral genome. Genome differentiation in evolution is achieved through tandem replication of repetitive sequences and their subsequent base sequences divergence. During the course of speciation, repeated sequence families may have diverged and thus increased mismatches in DNA sequence organization. On the other extreme, while some sequences are retained in a number of plant species they are completely eliminated from others. Such sequences become species-specific. It is postulated that amplification, elimination, rearrangements and mutations of repetitive sequences must have played a role in the differentiation of genomes in speciation (Hoang-Tang *et al*., 1991). The molecular mechanism responsible for DNA amplification is not clear. Earlier workers have suggested errors in replication as a mechanism (Schopf, 1981). Britten and Kohne (1968) have called the process "saltatory replication" of base sequences. Others have suggested by slippage mechanism combined with point mutations, unequal crossover and recombinational events (Tautz *et al*., 1986; Levinson and Gutman, 1987). The results of this investigation suggest that DNA sequences homologous to E-1800 fraction may be present in different copy number in related species and homologous sequences have undergone substantial base sequence divergence 'in evolution.

The taxonomical classification of *Lathyrus* species based on external morphology is not precise. However, species included in the subgeneric section *Lathyrus* show several morphological characters that are partially correlated. *L. tingitanus* is included in this section, Species of section *Lathyrus* are therefore expected to show a greater similarity in the distribution of restriction fragments than species included in other subgeneric sections. Similarity indices were calculated by comparing the pattern of distribution of satellite DNA restriction fragments in *L. tingitanus* with the species included in section *Lathyrus* and other subgeneric sections. Species included in other subgeneric sections (*Linearicarpus*, *Nissolia* and *Clyrnenum*) are taxonomically much diverged from species of section *Lathyrus*. Correspondingly the estimated mean similarity indices are lower than that estimated for species in section *Lathyrus*. In genus *Lathyrus* the distribution of 2C nuclear DNA amount is discontinuous. Species cluster into DNA groups, the group means themselves are separated by a regular DNA interval of approximately 4 pg. This grouping occur independently of the taxonomical affinities between species and it is viewed as an expression of the intrinsic constraints underlying genome organization. Similarity indices were calculated for the 5 DNA groups as before. The 5 group do not show significant differences in their mean values. The results would suggest that species having similar nuclear DNA amounts are not necessarily similar in the restriction profiles of the satellites DNA.

The 17 varieties of *L. sativus* showed very little variation in the restriction profiles of DNA sequences homologous to the satellite DNA. This confirms that within a species the satellite DNA sequences are fairly homogeneous in base sequence composition.

Acknowledgements

I thank Dr. R. K. J. Narayan for his expertise, generosity in spending time and illuminating suggestion during this investigation. Seeds provided by Dr. H. I. T Khawaja of Agriculture Research Institute Islamabad, Pakistan and Dr. Campbell of Agriculture Canada Research Station, Minitoba Canada are greatly appreciated.

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